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Effect of inhaled KP-496, a novel dual antagonist of the cysteinyl leukotriene and thromboxane A_2 receptors, on a bleomycin-induced pulmonary fibrosis model in mice

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ABSTRACT

Cysteinyl-leukotrienes (cysLTs) and thromboxane A_2 (TXA_2) are important mediators in inflammatory lung diseases such as bronchial asthma and idiopathic pulmonary fibrosis (IPF). We examined the effects of inhaled KP-496, a novel dual antagonist of the cysLTs and TXA_2 receptors, on bleomycin-induced IPF in mice. Mice were intravenously injected bleomycin on day 0, and 0.5% of KP-496 was inhaled twice a day (30 min/time) for the entire experimental period. The effects of KP-496 were evaluated by the number of infiltrated cells in bronchoalveolar lavage fluid (BALF), hydroxyl-L-proline content in the lung, and histopathology. Analyses of BALF on days 7 and 21 revealed that inhaled KP-496 significantly decreased total cell numbers, macrophages, neutrophils, and eosinophils on both days. KP-496 significantly decreased hydroxyl-L-proline content in the lung on day 21. Histopathological analyses of lungs on day 21 demonstrated that KP-496 significantly suppressed inflammatory and fibrotic changes. Our results suggested that the suppression of cysLTs and TXA_2 pathways by KP-496 could control airway inflammation and pulmonary fibrosis, and that KP-496 could be a new therapeutic agent for lung diseases with inflammation and fibrogenesis such as IPF and chronic obstructive pulmonary disease.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is the commonest interstitial pneumonia of unknown origin, and one of the most aggressive interstitial lung diseases. IPF is characterized by progressive alveolar inflammation, fibroblast proliferation, and collagen deposition [1,2]. The molecular mechanisms of IPF remain to be elucidated, but many studies suggest that some pro-inflammatory mediators, growth factors, and infiltrating cells may be involved in IPF development [3–6]. Currently, IPF is a progressive and fatal disease and effective treatments are not available.

Bleomycin (BLM)-induced lung injury in rodent is a well characterized model with similar pathological changes to human IPF. It is a commonly used *in vivo* model employed to estimate the anti-fibrotic potential of a therapeutic procedure [6,7]. Recent studies suggested that lipid mediators derived from arachidonic acid such as leukotrienes (LTs) and thromboxanes (TXs) are associated with the development of pulmonary fibrosis induced by BLM [8–14]. Therefore, a preventing the actions of these lipid mediators may be beneficial to the treatment of fibrotic lung diseases.

Cysteinyl-leukotrienes (cysLTs; LTC_4, D_4, and E_4) are thought to be the most important mediators in inflammatory lung diseases such as bronchial asthma. It is reported that cysLTs are overproduced in asthma and induce bronchial smooth muscle contraction, mucus hypersecretion, and eosinophilic inflammation [15,16]. Moreover, numerous reports demonstrated that cysLTs have a direct effect on fibroblasts with respect to migration, proliferation, and matrix protein synthesis [17–19]. Considering the physiological roles of cysLTs in inflammatory lung diseases, they may contribute to the induction/progression of IPF.

Similarly, TXA_2 is known to increase pulmonary vascular permeability and induce lung inflammation [20,21]. Cruz-Gervis et al. demonstrated that the decreased ratio of prostacyclin synthesis to TX synthesis is associated with the development of pulmonary fibrosis, and suggested that TXA_2 is also a target molecule for IPF treatment [22].

We developed a novel dual antagonist for LTD_4 and TXA_2 named KP-496 [23–27]. KP-496 was originally developed as an

Abbreviations: LTs, leukotrienes; TXA_2, thromboxane A_2; IPF, idiopathic pulmonary fibrosis; BALF, bronchoalveolar lavage fluid; BLM, bleomycin; MC, methylcellulose; H&E, hematoxylin and eosin.

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anti-asthmatic agent, and is currently under clinical development as a dry powder inhaler for asthma treatment and nasal spray for allergic rhinitis in Japan. The antagonistic activities of KP-496 for LTD_4 and TXA_2 are comparable with the launched respective antagonists [23]. Our other study revealed that inhaled KP-496 suppressed not only respiratory function but also airway inflammation in guinea pig allergic asthmatic models [27]. Considering our previous results and the contributions of LTs and TXA_2 to IPF pathology, a therapeutic effect of KP-496 on IPF could be expected. In the present study, we evaluated the therapeutic effect of inhaled KP-496 on a BLM-induced model of IPF in mice.

2. Materials and methods

2.1. Animals

Male ICR mice (9 weeks old, 33–42 g) were purchased from Japan Charles River (Yokohama, Japan). The animals had free access to standard laboratory feed and water in an air-conditioned room at 22 ± 1 °C with a relative humidity of 60%. The study protocol followed the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animal Science 1987. The protocol was approved by the Experimental Animal Research Committee at the Central Research Laboratories of Kaken Pharmaceutical Co., Ltd.

2.2. Materials

Bleomycin hydrochloride (Bleo for inj. 30 mg; Nippon Kayaku, Tokyo, Japan). Prednisolone, Giemsa solution, chloramine T, p-dimethyl-aminobenzaldehyde, and 2-methoxyethanol were purchased from Nacalai Tesque, Kyoto, Japan. Hematoxylin–eosin (H&E) solution and Azan solution were purchased from Muto Pure Chemical, Tokyo, Japan. KP-496 was synthesized by Kaken Pharmaceutical Co., Ltd. and dissolved in 1 mol/L of sodium hydroxide solution. This solution was diluted with physiological saline and adjusted to pH 7.5.

2.3. BLM-induced model of IPF

BLM solution (15 mg/mL) was intravenously injected into mice (0.1 mL/10 g of body weight) on day 0. Mice were sacrificed on day 7 or 21. They were exposed to an aerosol of KP-496 solution (0.5%) for 30 min using a pressure nebulizer (Pari, GmbH, Starnberg, Germany) 1 h before and 3 h after BLM-injection on day 0. From day 1 to the day before sacrificed, mice were exposed to KP-496 (0.5%) for 30 min morning and evening. In this study, when a mouse was exposed to aerosol of 0.5% of KP-496 for 30 min, the nominal dose of KP-496 calculated from concentration in the lung was about 20 μg/kg. This dosage was about 1.4 times higher than previous studies that used guinea pigs [24,27]. Prednisolone was suspended in 0.5% methylcellulose solution (0.5% MC) and administered orally 1 h before and 3 h after BLM-injection on day 0 (5 mg/kg). From day 1 to the day before sacrifice, prednisolone was orally administered every morning. Mice of the control group were exposed to physiological saline or orally administered 0.5% MC. Mice injected with physiological saline instead of BLM were considered to be non-IPF induced controls.

2.4. Bronchoalveolar lavage fluid (BALF) study

On days 7 and 21, mice were anesthetized by intraperitoneal injection of pentobarbital (30 mg/kg). The trachea was cannulated and the left bronchus was ligated. The right airway lumen was washed thrice with physiological saline. BALF from each mouse was pooled in a plastic tube and centrifuged (150×*g*) at 4 °C for 10 min. Cell pellets were resuspended in 200 μL of physiological saline. The total number of cells was counted using an automated cell counter (CDA-500, Sysmex, Kobe, Japan). A differential cell count was taken on a smear prepared by cytocentrifugation (Cytospin II; Shandon, UK) and stained with Giemsa solution (based on standard morphological criteria).

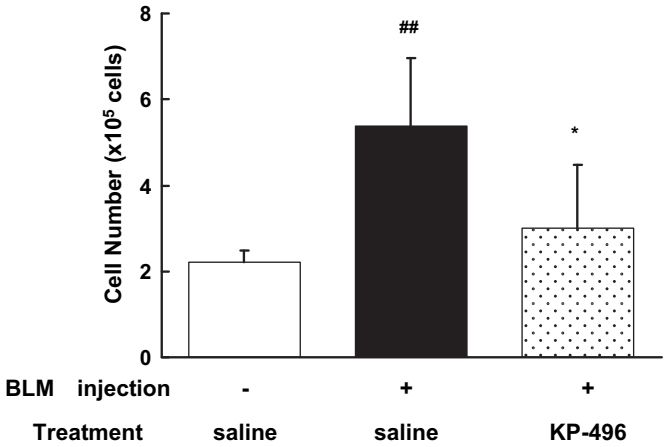

Fig. 1. Effect of inhaled KP-496 on bleomycin (BLM)-induced acute lung inflammation. On day 7 after BLM-injection, the trachea of mice was cannulated and the left bronchus was ligated. The right airway lumen was washed thrice with physiological saline. Cell pellets were resuspended in 200 μL of physiological saline and the total number of cells counted using a particle calculation analyzer. Results are mean ± SD for 8 mice. ##, $p < 0.01$ compared with saline-injected, saline-treated group (Student's *t*-test). *, $p < 0.05$ compared with BLM-injected, saline-treated group (Student's *t*-test).

2.5. Measurement of hydroxyl-L-proline content in lung

After recovery of BALF on day 21, the right lobe of the lung of each mouse was removed and lyophilized. Hydrochloric acid (6 mol/L) was added to the lyophilized lung and heated at 110 °C for 24 h. After adjusting the pH to 7–9, 0.5 mol/L borate buffer, potassium chloride, and 0.2 mol/L chloramine T solution were

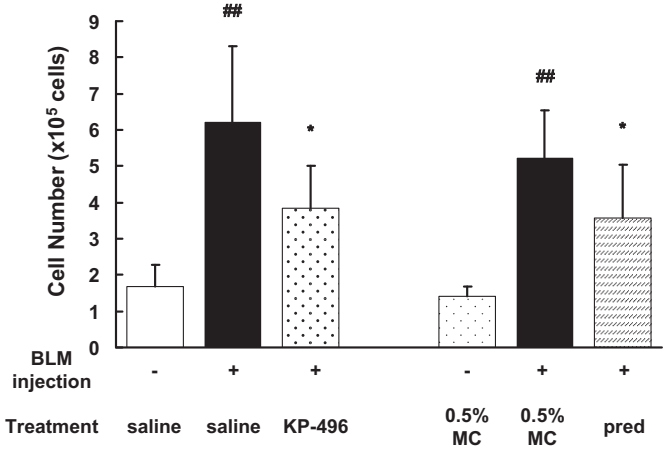

Fig. 2. Effects of inhaled KP-496 and oral prednisolone on BLM-induced chronic lung inflammation. On day 21 after BLM-injection, the trachea of mice was cannulated and the left bronchus was ligated. The right airway lumen was washed thrice with physiological saline. Cell pellets were resuspended in 200 μL of physiological saline and the total number of cells counted using a particle calculation analyzer. Results are mean ± SD for 7 or 8 animals. ##, $p < 0.01$ compared with saline-injected, saline-treated group (Student's *t*-test). *, $p < 0.05$ compared with BLM-injected, saline/0.5% MC-treated group (Student's *t*-test).